<Original>Studies on Digestive System of Termites : II. Properties of Carbohydrolases of Termite Coptotermes formosanus SHIRAKI

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Original

Studies on Digestive System of Termites*

II. Properties of Carbohydrolases of Termite *Coptotermes formosanus* SHIRAKI

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and Tetsuo KOSHIJIMA**

Abstract——The digestion of carbohydrates in termite *Coptotermes formosanus* SHIRAKI was demonstrated to be carried out by co-operative action of carbohydrolases originated from both termites and protozoa. Cellulases and β-glycosidases were mainly ascribed to protozoa, while α-glucosidase and α-mannosidase are to termites. Gel filtration was shown to provide a useful purification method for carbohydrolases of termites, since the molecular weights of cellulases are lower than those of aryl-glycosidases. The effects of temperature, pH, metal ions and organic reagents including surfactants on the partially purified enzyme preparations toghether with isoelectric points (pI) were also determined. These results provided gross characteristics of carbohydrolases which may be useful for further elucidation of the digestive system of *C. formosanus* and for application of the enzymes to saccharification of lignocellulosic materials.

1. Introduction

The protozoan's symbiotic role in lower termites[1] has been a basis for elucidating digestive system of termites. Trager[2] demonstrated the cellulolytic ability of a protozoon isolated from *Zootermopsis angusticollis*. Hungate[3] has reported that termites (*Zootermopsis* sp.) containing protozoa show cellulase activity which is completely lost after removal of protozoa by heat-treatment. The complete dependence of digestive system of cellulose on protozoa in lower termites is, however, shown to be doubtful based on newly presented evidences[4~8]. In our country, Yokoe[9] firstly presented a new evidence that *Leucotermes* (*Reticulitermes*) *speratus* secreted its own cellulase independently of any enzymes produced by the protozoa. Yamaoka and Nagatani[10] also reported that carboxymethylcellulase (CMCase) was secreted from the salivary gland of *R. speratus* while Avicelase was produced by protozoa. They proposed that cellulose was firstly decomposed by protozoan cellulase followed by termites own CMCase. These investigations were carried out with *R. speratus* which were distributed through the whole country of Japan except high mountains and extremely cold regions. In the Pacific warm district of Japan including Shikoku, Kyushu and

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Okinawa islands, however, the other species of termite, *Coptotermes formosanus*, was widely distributed and caused more severe damage to wooden buildings than *R. speratus*. This prompted us to elucidate the digestive system of *C. formosanus*. Mauldin *et al.*[8] reported that a large-size protozoon (*Pseudotrichonympha grassi*) was neccessary for normal cellulose catabolism in *C. formosanus*. Carter *et al.*[11] showed that *P.* grassi was seriously affected by exposure of *C. formosanus* with heart wood from 21 wood species. In the previous study[12], we fed termite workers of *C. formosanus* with various carbohydrate diets and found that *P. grassi* participated in the decomposition of cellulase and termites themselves were able to utilize amylose, cellobiose, maltose, glucose and fructose without aid of protozoa.

In the present study, we intended to compare the activities of carbohydrolases of workers with those of soldiers, clarify the effects of defaunation on carbohydrolases, shed light on the location of carbohydrolases in termites, and characterize the properties of partially purified cellulase from worker termites of *C. formosanus*.

2. Experimental

2.1 *Materials and methods*

Unless otherwise specified, materials and methods were the same as those described in the previous papers[12,13]. Enzyme activities were calculated by subtracting the absorbancies due to sole enzyme and substrate. One unit of aryl-glycosidase activity is defined as the amount of enzyme which liberates 1 μmol of *p*-nitrophenol/min under the condition described previously[13]. One unit of polysaccharase activity is defined as the amount of enzyme which liberates 1 μmol of monosaccharide under the condition described previously[13].

2.2 *Defaunation of worker termites*

Three types protozoa have been shown to be present in the gut of *C. formosanus*: large size (*Pseudotrichonympha grassi*), middle size (*Holomastigotoides hartmanni*) and small size (*Spirotrichonympha leidyi*) protozoa[12]. In this study, three different defaunation treatments were carried out: 1) Starvation; 2) Heat treatment; and 3) Oxygen treatment. Starvation experiment was carried out at 26°C for 192 hr, by a modification of the method of Cleveland[14]. By this treatment *P. grassi* was eliminated but *H. hartmanni* and *S. leidyi* were maintained in 50% and 85% of surviving worker termites, respectively. Heat treatment was carried out at 36 ± 1°C for 60 hr, by a modification of the method of Cleveland[14]. By this treatment *P. grassi* was also completely eliminated but *H. hartmanni* and *S. leidyi* were maintained in 55% and 85% of surviving worker termites, respectively. Oxygen treatment was carried out by exposure of workers to pure oxygen gas at 1 Kg/cm^2 for 40 hr, by a modification of the method of Bready and Friedman[4]. All protozoa were eliminated from termites

by this treatment.

2.3 *Location of enzymes*

Four hundred termites in 3 ml of 0.05 M sodium acetate buffer (SAB), pH 4.8, were sonicated at 5°C for 4×30 sec. The homogenate was centrifuged to obtain clear supernatant. The precipitate was washed twice with 0.5 ml of SAB by centrifugation. The supernatant and washings were pooled, filled up to 4 ml, and used as a standard enzyme solution. Four hundred termites were separated into head and body portions which were sonicated as described above. The gut of the termites was obtained by pulling anus out with tweezers from four hundred workers and also sonicated. The positions of head, body and gut are shown in Fig. 1.

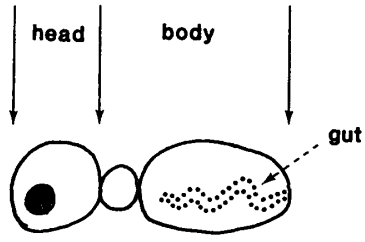

Fig. 1. Schematic illustration of the positions of head, body and gut of termites.

2.4 *Isolation of crude enzymes*

Twenty-five mililiters of 0.05 M SAB was added to 5 g of worker termites and sonicated at 5°C for 6×30 sec. The homogenate was centrifuged at 15,000×g at 5°C for 20 min to remove insoluble materials. This crude enzyme solution was treated with ammonium sulfate. The precipitate formed was recovered by centrifugation, washed with ammonium sulfate solution and solubilized in 3 ml of SAB. Insoluble material in SAB was removed by centrifugation.

2.5 *Purification of enzyme*

Purification of cellulase was carried out by a combination of ammonium sulfate fractionation, gel filtration, and isoelectric focusing. These operations were carried out at 5°C. Gel filtration experiments were carried out on columns of Sephadex G-50 (2.5×53.5 cm), Sephadex G-100 (1.0×57 cm) and Sepharose 4B (1.0×50 cm) preequilibrated with 0.025 M sodium phosphate buffer (SPB), pH 6.8. Six-ml fractions were collected for Sephadex G-50 while 1 ml for Sephadex G-100 and Sepharose 4B.

3. Results and Discussion

3.1 *Carbohydrolases of workers and soldiers*

Activities of carbohydrolases of workers were compared with those of soldiers

Table 1-(a). Total Activities of Carbohydrolases per 1000 Worker Termites*

Enzymes	Position of termite			
	Whole	Head	Body	Gut
α-galactosidase	147.5	0	74.7	100.8
β-galactosidase	74.1	13.9	45.8	88.4
α-glucosidase	108.6	3.3	30.3	45.4
β-glucosidase	568.2	54.3	374.1	342.6
α-mannosidase	231.6	49.2	94.2	54.2
β-mannosidase	267.9	3.2	211.9	139.2
α-xylosidase	20.7	1.8	8.0	6.5
β-xylosidase	64.9	28.0	52.6	59.0
α-arabinosidase	22.7	6.5	9.2	20.1
Avicelase	97.0	3.3	14.7	3.3
CMCase	379.4	74.8	286.6	362.7
Xylanase	654.9	16.4	503.8	574.1
Arabinoxylanase	493.3	10.0	376.1	44.3
Galactanase	141.4	20.3	88.3	76.3
Mnnnanase	711.8	184.5	369.7	191.7
Arabanase	158.6	43.2	235.3	115.5
Amylase	506.0	17.5	493.6	110.0
Pectinase	381.1	0	401.6	73.0
Dextranase	107.1	0	27.4	9.8
Ball-milled pine wood degrading enzyme	223.7	25.0	360.5	169.9
Ball-milled beech wood degrading enzyme	229.4	14.2	318.0	63.7
Filter paper degrading enzyme	63.0	3.6	94.6	27.2

* Units/1000 individuals.

(Tables 1 and 2). Both workers and soldiers had all activities of enzymes tested, indicating that *C. formosanus* could digest various cabohydrates. This may be one of the reasons why *C. formosanus* causes serious damage to wooden buildings. Total activities of polysaccharases and β-glycosidases of workers were 1.3–7.6 fold and 1.9–4.1 fold higher than those of soldiers, respectively. Regarding specific activities these values were 1.2–6.6 for polysaccharases and 1.3–3.6 for β-glycosidases. No consistent relationship was, however, obserbed in the activities of α-glycosidases. Activities of α-glucosidase and α-xylosidase of workers were similar to or rather smaller than those of soldiers, whereas activities of the other α-glycosidases were higher than those of soldiers. Activity of α-arabinosidase of workers was sometimes lower than that of soldiers. Note that soldiers had appreciable amount of polysaccharases and aryl-glycosidases. This seems to be peculiar since soldiers cound not eat and their diets are completely dependent on workers. Since soldiers also have protozoa, although the amount of which is much lower than in workers, the activities of the polysaccharases

Table 1-(b). Total Activities of Carbohydrolases per 1000 Soldier Termites*

Enzymes	Position of termite			
	Whole	Head	Body	Gut
α-galactosidase	69.6	0	58.3	38.5
β-galactosidase	39.5	1.2	45.9	45.3
α-glucosidase	105.5	1.2	141.7	58.9
β-glucosidase	216.0	5.9	178.9	32.4
α-mannosidase	58.6	5.3	68.3	54.4
β-mannosidase	64.8	5.2	79.5	83.8
α-xylosidase	20.0	0.6	31.9	0
β-xylosiddse	25.0	1.2	57.2	56.6
α-arabinosidase	13.6	0.8	6.7	19.0
Avicelase	23.9	7.0	32.0	2.1
CMCase	147.3	39.4	271.5	130.7
Xylanase	273.1	39.4	353.4	202.6
Arabinoxylanase	223.7	23.2	245.9	138.3
Galactanase	79.3	34.8	105.0	7.6
Mannanase	289.4	49.8	253.5	196.1
Arabanase	120.9	81.2	105.0	18.5
Amylase	102.9	58.0	78.1	1.1
Pectinase	50.2	9.3	33.3	1.1
Dextranase	35.6	0	23.0	0
Ball-milled pine wood degrading enzyme	100.7	16.2	163.9	55.6
Ball-milled beech wood degrading enzyme	97.4	67.3	124.2	54.5
Filter paper degrading enzyme	19.7	16.2	35.5	4.4

* Units/1000 individuals.

and aryl-glycosidases in soldiers may be partially ascribed to protozoa. The remaining activities are due to soldiers themselves. Presence of high α-glucosidase activity in termites may be neccessary for digesting trehalose and glycogen or glycogen-like carbohydrates. Present results also suggest that some kinds of polysaccharases and aryl-glycosidases are indispensable for maintaining life of termites.

3.2 *Location of carbohydrolases in termites*

For clarifying the location of carbohydrolases in termites, enzyme activities of three portions of termites, *i.e.* head, body and gut, were compared. As shown in Tables 1 and 2, the majority of enzyme activities existed in body especially in gut. Only a small but appreciable amount of enzyme activities was present in head. Presence of 15–20% of the total CMCase activity in head of workers is in agreement with the results of Yamaoka and Nagatani[10] who found that two cellulases were present in *R. speratus*: one in the salivary glands and the other from protozoa.

In the present study, we demonstrate that not only cellulase but also the other poly-

Table 2-(a). Specific Activities of Carbohydrolases of Worker Termites*

Enzymes	Position of termite			
	Whole	Head	Body	Gut
α-galactosidase	3.4	0	4.7	11.8
β-galactosidase	2.0	1.6	2.9	10.3
α-glucosidase	3.1	0.6	1.9	5.3
β-glucosidase	19.9	6.7	23.7	39.9
α-mannosidase	8.1	6.3	6.0	6.3
β-mannosidase	9.4	0.4	13.4	16.2
α-xylosidase	0.7	0.2	0.5	0.8
β-xylosidase	2.3	5.1	3.3	6.9
α-arabinosidase	3.1	0.7	1.1	2.3
Avicelase	3.4	1.0	1.8	0.9
CMCase	13.3	13.5	29.1	6.5
Xylanase	23.0	3.0	45.7	66.9
Arabinoxylanase	2.6	1.9	25.9	51.7
Galactanase	5.0	3.7	6.8	8.9
Mannanase	25.0	14.9	20.6	22.4
Arabanase	5.6	3.9	5.2	13.5
Amylase	17.8	3.2	17.3	17.3
Pectinase	13.4	1.0	10.4	11.1
Dextranase	3.8	0.7	2.9	2.1
Ball-milled pine wood degrading enzyme	6.6	4.5	22.9	19.8
Ball-milled beech wood degrading enzyme	6.7	2.6	20.2	27.4
Filter paper degrading enzyme	1.8	0.7	6.0	3.2

* Units/mg protein.

saccharases and aryl-glycosidases are present in head portion of termites. Since worker termites of *C. formosanus* could not live on hemicellulosic carbohydrates as sole diet as previously pointed out[12] and cellulolytic enzymes are important in enzymatic saccharification of woody plants, we did not in this study further mentioned polysaccharases other than cellulases.

3.3 *Effects of defaunation on carbohydrolase activities*

In order to elucidate the role of protozoa in termites, the enzyme activities were determined after the protozoa were removed from termites by treatment with oxygen, heat and starvation. A large size protozoon (*P. grassi*) was completely lost by all treatments. Middle size (*H. hartmanni*) and small size (*S. leidyi*) protozoa still remained after starvation and heat treatments, but completely lost by oxygen treatment. The results shown in Table 3-(a) indicate marked decrease in activities of cellulases and β-glycosidases which were in turn due to large size protozoon (*P. grassi*). The activities of α-glucosidase and α-mannosidase were higher than those of normal worker

Table 2-(b). Specific Activities of Carbohydrolases of Soldier Termites*

Enzymes	Position of termite			
	Whole	Head	Body	Gut
α-galactosidase	1.7	0	2.6	2.7
β-galactosidase	1.5	0.3	3.8	3.2
α-glucosidase	3.9	0.3	4.3	4.2
β-glucosidase	8.8	1.3	10.2	23.3
α-mannosidase	2.4	1.2	3.9	3.9
β-mannosidase	2.6	1.1	4.5	5.9
α-xylosidase	0.7	0.1	0.4	0
β-xylosidase	1.0	1.3	2.3	4.0
α-arabinosidase	1.7	0.2	1.6	2.3
Avicelase	1.0	0.2	0.9	0.6
CMCase	6.0	4.1	15.4	35.6
Xylanase	11.1	4.9	20.1	55.2
Arabinoxylanase	9.1	2.9	14.0	37.7
Galactanase	3.2	1.8	6.0	2.1
Mannanase	11.8	4.8	14.4	13.4
Arabanse	4.9	2.4	4.0	5.1
Amylase	4.2	3.1	4.4	0.3
Pectinase	2.0	1.0	1.3	0.3
Dextranase	1.5	0.3	1.3	0
Ball-milled pine wood degrading enzyme	4.1	3.3	9.3	15.4
Ball-milled beech wood degrading enzyme	4.0	14.6	7.1	14.8
Filter paper degrading enzyme	0.8	3.5	2.0	1.2

* Units/mg protein.

termites. This clearly indicates that the activities of these enzymes are not dependent on protozoa but consequently ascribed to termites. The marked decrease in β-glucosidase and cellulase activities after oxygen treatment indicate involvement of both middle and small size protozoa.

In the gut, all enzymatic acitivities other than α-glucosidase and α- and β-mannosidases clearly decreased by starvation and heat treatments. In addition, activity of β-glucosidase further decreased by oxygen treatment (Table 3-(b)). In order to ascertain the effects of defaunation treatments on termites, the activities in the residues after removal of gut were also examined. As shown in Table 3-(b), the loss of enzyme activity was detected only in α-galactosidase and CMCase. Oxygen treatment did not induce substantial decrease in enzyme activity. From these results, it may suffice to conclude that these defaunation treatements did not seriously affect termites.

From the results described above, it is concluded that α-glucosidase and α-mannosidase are independent with protozoa but β-glucosidase is dependent on all three

Table 3-(a). Effects of Defaunation on Relative Total Activities of Carbohydrolases in Whole Worker Termites*

Enzymes	Heat treatment	Oxygen treatment	Starvation treatment
α-galactosidase	61.2	54.0	30.0
β-galactosidase	45.8	42.3	47.0
α-glucosidase	117.8	112.3	121.1
β-glucosidase	38.3	39.5	38.3
α-mannosidase	116.8	104.2	107.4
β-mannosidase	55.0	62.9	67.5
α-xylosidase	80.0	90.5	84.2
β-xylosidase	39.3	40.6	55.3
α-arabinosidsae	44.1	27.8	59.9
Avicelase	41.0	16.3	64.3
CMCase	37.0	14.2	46.4

* Each value was given as a relative percentage of the total activity/1000 termites survied after defaunation to that obtained before defaunation.

Table 3-(b). Effects of Defaunation on Relative Total Activities of Carbohydrolases in the Gut portion of Worker Termites*

Enzymes	Heat treatment	Oxygen treatment	Starvation treatment
α-galactosidase	28.9 (79.3)**	36.3 (76.7)**	38.3 (36.7)**
β-galactosidase	62.7 (147.6)	27.4 (167.1)	66.4 (98.8)
α-glucosidase	200.3 (203.3)	77.9 (146.4)	198.5 (132.0)
β-glucosidase	59.0 (100.0)	18.5 (119.9)	43.0 (135.1)
α-mannosidase	164.8 (120.8)	70.2 (91.7)	139.6 (116.7)
β-mannosidase	107.2 (100.0)	62.5 (87.0)	95.8 (79.7)
α-xylosidase	33.3 (137.1)	33.3 (98.3)	66.7 (143.7)
β-xylosidase	56.9 (109.9)	17.0 (90.7)	15.9 (85.7)
α-arabinosidase	19.6 (N.D.)	11.7 (N.D.)	17.6 (N.D.)
Avicelase	25.6 (106.4)	42.2 (101.1)	19.8 (77.9)
CMCase	21.9 (77.2)	37.7 (76.1)	13.7 (77.2)

* Each value was given as a relative percentage of the total activity/gut from 1000 termites survived after defaunation to that obtained before defaunation.

** Each parenthesis represents relative activity of the residue remained after removal of gut from defaunated termite to that obtained before defaunation.

N.D.: not done.

protozoa and that the other enzymes are mainly related to large size protozoon. On account of cellulases in the whole termites, both Avicelase and CMCase activities decreased by removing large size protozoon and further decreased by removing middle and small size protozoa, indicating the participation of middle and small size protozoa in degradation of cellulose. This was further supported by the results

obtained with gut from workers. Alpha-glucosidase may be induced by defaunation treatment, indicating involvement in degradation of trehalose and glycogen or glycogen-like carbohydrates as previously suggested.

3.4 *Purification of carbohydrolases*

1) Fractionation with ammonium sulfate

The crude enzyme preparation was subjected to ammonium sulfate fractionation as shown in Fig. 2. Aryl-glycosidases and polysaccharases were precipitated from the slightly yellowish supernatant at 35–80% saturation with ammonium sulfate. Eighty two to 98% of aryl-glycosidases and 86–88% of cellulases were recovered in the precipitates at 50–65% and 50–80% saturations with ammonium sulfate, respectively.

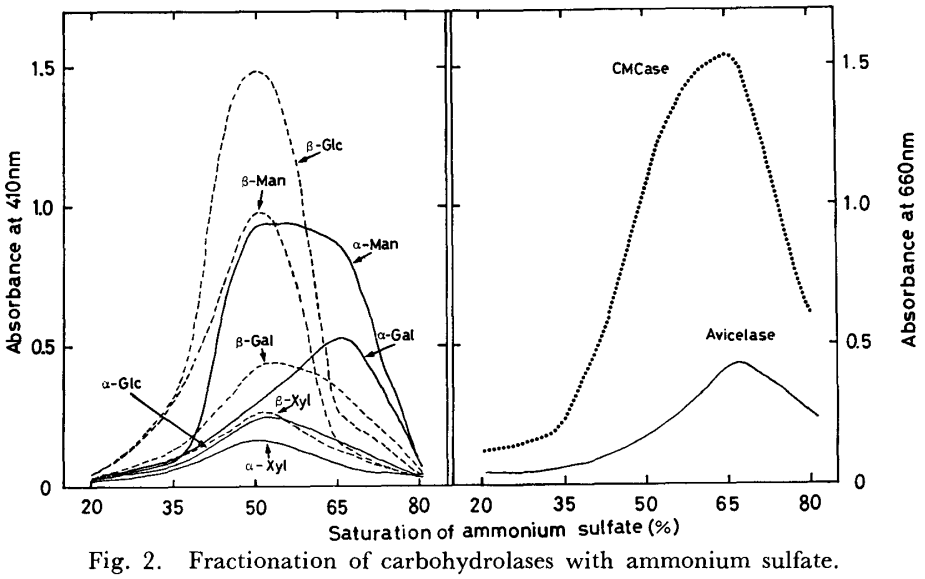

Fig. 2. Fractionation of carbohydrolases with ammonium sulfate. α-Glc=α-glucosidase, *etc.*

2) Gel filtration

Ten milligrams of the enzyme preparation at 35–80% saturation with ammonium sulfate was dissolved in 0.5 ml of SPB, and applied to Sephadex G-50 equilibrated with the same buffer. Almost all enzymes were eluted near void volume as shown in Fig. 3. A small amount of peptides which did not have enzyme activity was eluted near column volume. Thus Sephadex G-100 was used for enzyme separation. As shown in Fig. 4, two major fractions were detected. One fraction eluted just behind void volume contained aryl-glycosidases, but the other lower molecular weight fraction contained no activity. Cellulases such as CMCase and Avicelase were mainly eluted between these two fractions, but did not show distinctive peaks. These results indicate that gel filtration on Sephasex G-100 is effective for separation of cellulases from aryl-glycosidases. The molecular weights of CMCase and Avicelase were estimated

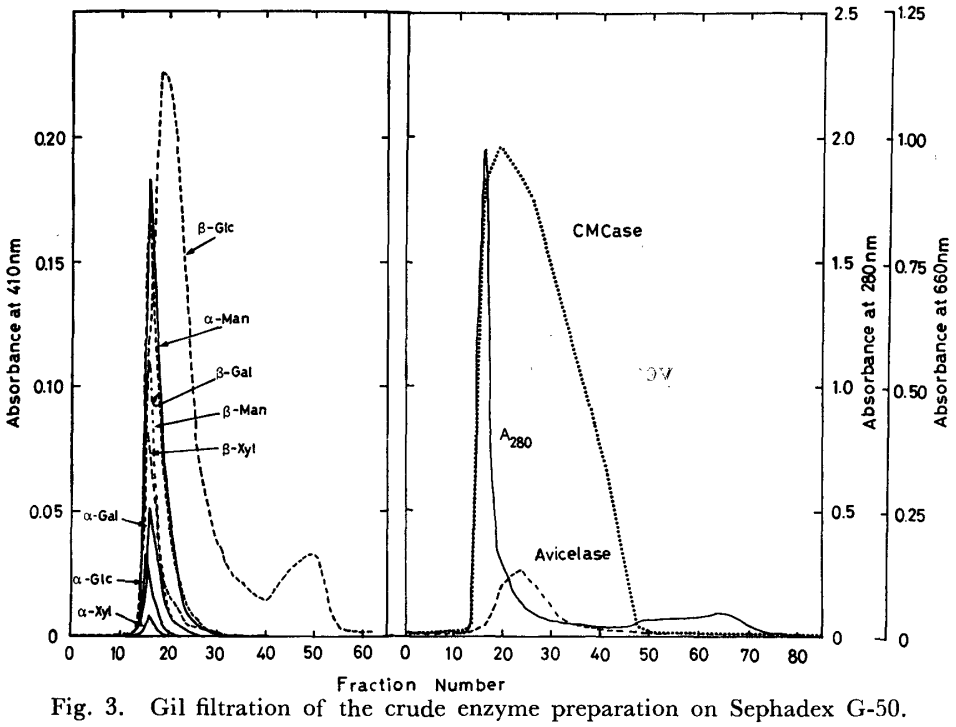

Fig. 3. Gil filtration of the crude enzyme preparation on Sephadex G-50. Abbreviation was the same as shown in Fig. 2.

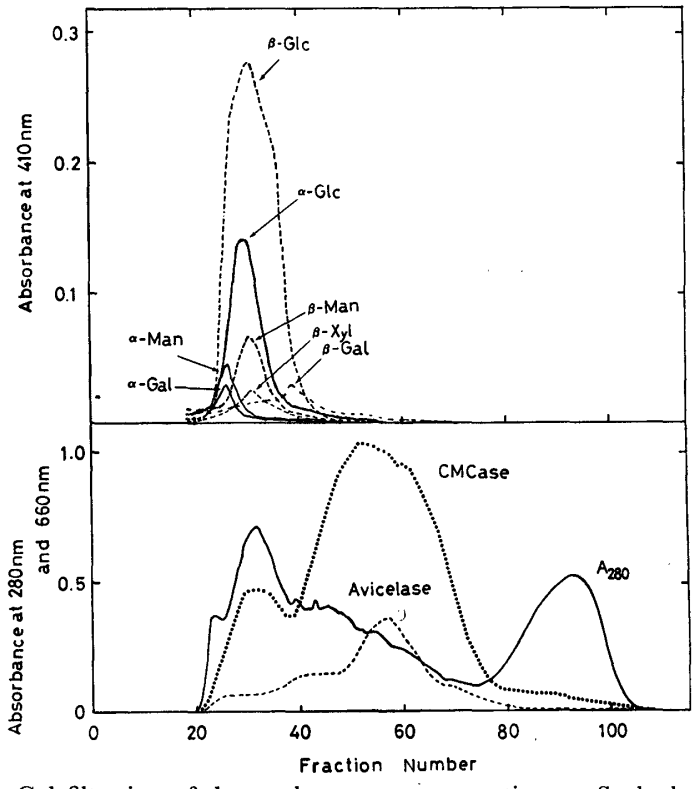

Fig. 4. Gel filtration of the crude enzyme preparation on Sephadex G-100. Abbreviation was the same as shown in Fig. 2.

to 32,000 and 27,000, respectively. The molecular weights of aryl-glycosidases were in the range from 43,000 to 72,000.

Finally the crude enzyme solution was subjected to gel filtration on Sepahrose 4B. As shown in Fig. 5, three peaks were detected. The first peak at the void volume having molecular weight higher than 2.0×10^6 did not have any enzyme activity. The major peak having molecular weight of 40,000–80,000 contained majority of aryl-glycosidases. The last peak having molecular weight of 20,000–40,000 were rich in cellulase.

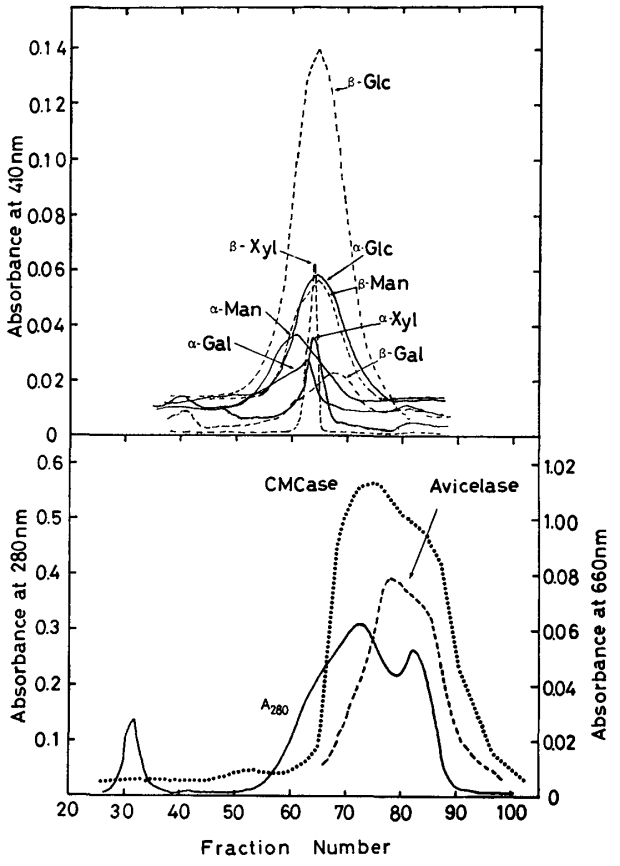

Fig. 5. Gel filtration of the crude enzyme preparation on Sepharose 4B. Abbreviation was the same as shown in Fig. 2.

When the crude enzyme solution prepared from soldiers was subjected to gel filtration, the elution profile and the distribution of enzyme activity were closely similar to those with worker termites except that the amount of the lower molecular weight contaminant in soldiers was somewhat higher than that of workers and that enzyme activities in soldiers were lower than those in workers.

Ion-exchange chromatography on DEAE-Sephadex A-50, cellulose affinity chromatography[17], and gause column chromatography[17] failed to further purify carbohydrolases of termites. Note that cellulases of termites have low affinity to crystal-

line cellulose in contrast to those originated from *Trichoderma* sp.[17]

When the activities of the partially purified carbohydrolases of termites are compared with those of the commercially available cellulase prepations, α-galactosidase, β-glucosidase, α- and β-xylosidases and CMCase activities of termites were lower than Onozuka R-10 and Cellulosin AC, whereas Avicelase, β-galactosidase and β-mannosidase activities of termites were intermediate between Onozuka R-10 and Cellulosin AC (Table 4). The α-glucosidase and α-mannosidase activities of termites were higher than those of the commercial enzyme preparations. These results may be useful for application of carbohydrolases of termites to saccharification of lignocellulosic materials.

Table 4. Activities of Carbohydrolases from Worker Termites and Commercially Available Cellulase Preparations*

Enzymes	Termites (workers)	Cellulase Onozuka R-10	Cellulosin AC
α-galactosidase	3.3	57.4	77.6
β-galactosidase	11.4	0.4	54.2
α-glucosidase	5.3	0.9	2.2
β-glucosidase	19.9	93.3	79.1
α-mannosidase	21.3	0.7	0.5
β-mannosidase	10.5	0.1	19.2
α-xylosidase	0.2	0.4	7.1
β-xylosidase	2.4	3.9	38.0
α-arabinosidase	0.7	21.7	18.5
Avicelase	3.4	8.3	1.7
CMCase	13.3	26.4	27.3

* Units/mg protein.

3.5 *Isoelectric focusing of carbohydrolases of worker termites*

The enzymes precipitated at 35–80% saturation with ammonium sulfate was deionized through Biogel P-2 and subjected to isoelectric focusing for the determination of pI as shown in Fig. 6. The pI values of β-glucosidase, α-galactosidase, β-galactosidase, α-mannosidase and β-mannosidase were 4.6, 4.6, 4.7, 4.2 and 4.3 respectively. There showed no clear peaks of α- and β-xylosidases, indicating that these enzymes were labile to this treatment. As for cellulase, Avicelase showed a broad peak having a maximum at pH 4.1, whereas CMCase showed a sharp single peak at pH 4.7. The pI of CMCase was, however, ovelapped with that of β-galactosidase.

3.6 *Effects of metal inons on the enzyme activity*

Effects of metal ions on the activities of carbohydrate-hydrolyzing enzymes were analyzed by incubation in the presence of 0.05 M of each metal salt. As shown in

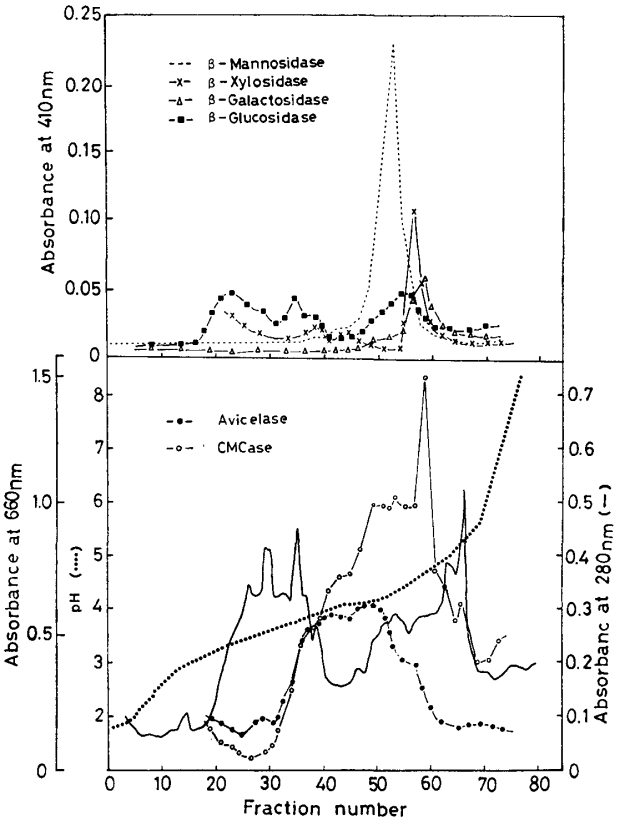

Fig. 6. Isoelectric focusing of the crude enzyme preparation deionized through Biogel P-2.

Table 5. Effect of Metal Ions on Enzyme Activity (Relative %)*

Metal ion	Aryl-glycosidases							Cellulases	
	α-Glc**	β-Glc	α-Gal	β-Gal	α-Man	β-Man	β-Xyl	Avicelase	CMCase
None	100	100	100	100	100	100	100	100	100
Na^{+}	57.1	104.4	90.9	107.6	81.8	69.9	141.7	113.6	90.4
Ni^{2+}	0	88.9	0	102.5	68.2	48.1	0	41.3	85.4
Cd^{2+}	42.9	42.2	9.1	30.4	63.6	48.1	0	46.3	9.4
Co^{2+}	57.1	95.6	9.1	101.3	104.5	69.0	150.0	29.5	72.0
Mn^{2+}	0	37.8	0	91.0	100.0	50.6	183.4	0	67.5
Fe^{2+}	0	8.9	0	6.3	0	13.0	0	0	0
Mg^{2+}	128.6	102.2	81.8	103.8	95.5	74.5	116.7	7.7	74.3
Ca^{2+}	214.3	97.8	109.1	112.7	104.5	73.6	83.3	12.2	62.5
Zn^{2+}	542.9	60.0	209.1	43.0	63.6	31.4	100.0	27.1	74.6
Hg^{2+}	328.6	2.2	9.1	0	0	27.2	0	0	4.3
Cr^{2+}	0	40.0	72.7	59.5	59.1	33.5	75.0	0	1.5
Cu^{2+}	128.6	84.4	372.7	92.4	464.6	100.0	900.0	13.8	155.5

* Each value was given as a relative percentage of the activity obtained in the presence of metal ion to that obtained in the native state.

** α-Glc=α-glucosidase *etc.*

Table 5, sodium ion slightly activate Avicelase but inactivate CMCase. Avicelase activity was markedly inhibited by divalent metal ions. CMCase activity was also inhibited by divalent metal ions but their effects were lesser than those observed in Avicelase. Beta-glucosidase activity was not inhibited by Na^{+} and Mg^{2+} ions, slightly inhibited by Ni^{2+}, Ca^{2+} and Cu^{2+} ions, but considerably inhibited by the other ions. The other enzyme activities showed different inhibition and activation against metal ions.

3.7 *Effects of organic reagents on the enzyme activity*

Effects of various reagents on the activities of carbohydrolases were analyzed by incubation in the presence of 0.05 M of each reagent. As shown in Table 6, both activities of Avicelase and CMCase were completely inhibited by *N*-bromosuccinimide (NBS). Avicelase was more labile than CMCase, since the former was completely inhibited by ethylenediaminetetraacetic acid (EDTA) and sodium lauryl sulfate (SDS), while the latter activity was still remained by these reagents. Beta-glucosidase activity, however, was stable for EDTA. These results indicate that a certain metal ions play an important role for maintaining the conformation of cellulase. Inhibitory effects of SDS might also be due to conformational change of enzyme proteins. However, 100% inhibition caused by NBS suggests that tryptophan residues may participate in the active center of enzyme proteins. CMCase activity was stable for thiol-reagents such as *N*-ethylmaleimide (NEM) and *p*-chloromercuribenzoate (pCMB), while 30.5% of Avicelase acitivity was inhibited by pCMB. Stability of cellulolytic enzymes toward

Table 6. Effect of Organic Reagents on Enzyme Activity (Relative %)*

Reagents	Aryl-glycosidases							Cellulases	
	α-Glc**	β-Glc	α-Gal	β-Gal	α-Man	β-Man	β-Xyl	Avicel-ase	CMC-ase
None	100	100	100	100	100	100	100	100	100
N-Ethylmaleimide	82.2	104.4	111.0	109.0	102.2	104.7	117.4	99.6	106.3
p-Chloromercuribenzoate	51.1	36.7	112.8	83.2	93.9	45.3	93.5	69.5	97.9
N-Bromosuccinimide	0	18.9	39.7	53.1	5.7	5.7	0	0	0
Etylenediamine teraacetate	27.3	107.6	28.6	113.3	90.0	83.7	100.0	0	15.3
Brij 35	110.3	110.6	82.2	113.3	98.4	103.0	97.8	101.1	75.9
Triton X-100	132.1	114.9	115.5	111.1	107.5	103.7	126.1	77.2	94.9
NoidetP-40	57.9	115.7	20.0	103.0	104.6	108.8	91.5	96.0	71.5
Tween 80	111.5	108.4	84.4	110.0	105.0	103.7	115.2	119.5	97.9
Cetyltrimethylammonium bromide	83.3	90.0	28.9	98.9	95.1	44.6	84.8	11.3	62.3
Sodium lauryl sulfate	84.6	27.2	0	7.8	44.0	0.7	10.9	0	10.9
Sodium deoxycholate	109.0	110.1	22.2	78.9	97.6	98.0	108.7	77.2	94.9

* Each value was given as a relative percentage of the activity obtained in the presence of reagent to that obtained in the native state.

** α-Glc=α-glucosidase *etc.*

non-ionic surfactants indicates the possibility of their usage for enzyme extraction and purification.

3.8 *Effects of pH on the enzyme activity*

The pH dependence of carbohydrolases was examined by incubation in 0.2 M MacIlavin buffer ranging pH from 2.2 to 8.0. As shown in Fig. 7, all aryl-glycosidases except α-mannosidase which had optimal pH at 4.5, showed the highest activity at about 5.0. Cellulase activity, however, showed maximum at pH 6.0. In the case of *R. speratus*, Yokoe reported that the optimal pH of 5.5 for CMCase[9]. Yamaoka and Nagatani, however, reported that both CMCase and filter paper-degrading enzyme of *R. speratus* showed maximal activities at pH 6.5[10].

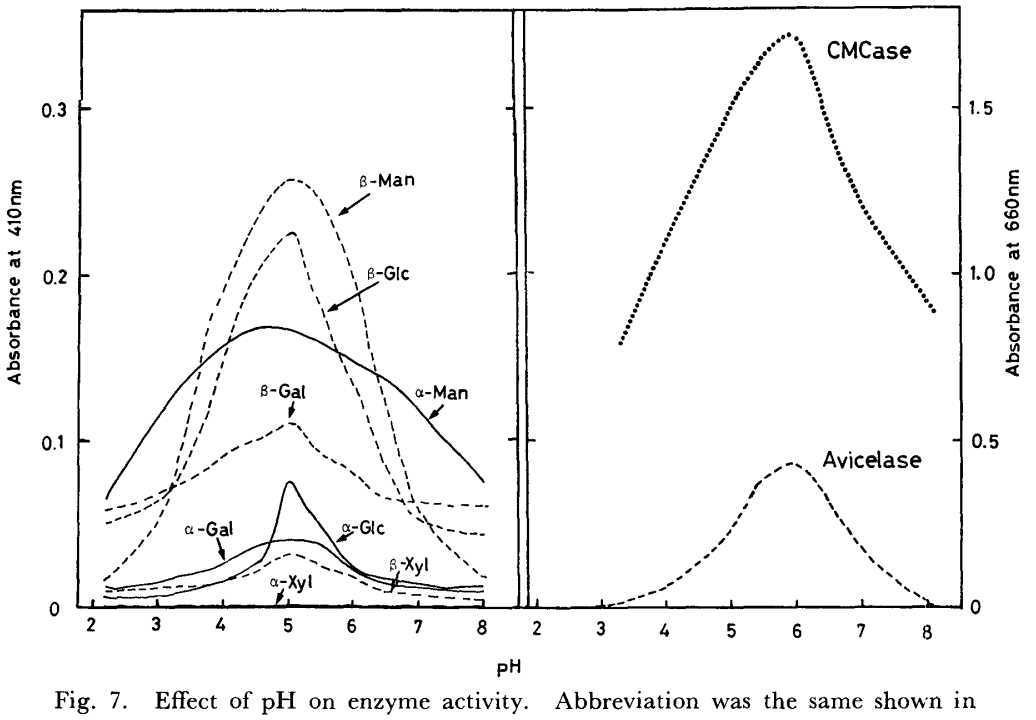

Fig. 7. Effect of pH on enzyme activity. Abbreviation was the same shown in Fig. 2.

3.9 *Effects of temperature on the enzyme activity*

The temperature dependence of carbohydrolases was examined by incubation at various temperatures for 10 min. As shown in Fig. 8, the optimal temperatures for α-galactosidase, β-galactosidase, α-glucosidase, β-glucodidase, α-mannosidase, β-mannosidase, α-xylosidase and β-xylosidase were 60°C, 50°C, 45°C, 45°C, 60°C, 45°C, 40°C and 40°C, respectively. The optimal temperatures for cellulases were 40°C for Avicelase and 50°C for CMCase, respectively.

In conclusion, present study provided gross properties of carbohydrolases of termite *C. formosanus* which may be clue to further elucidate the digestive system of *C. formosanus* and apply enzymes to saccharification of lignocellulosic materials.

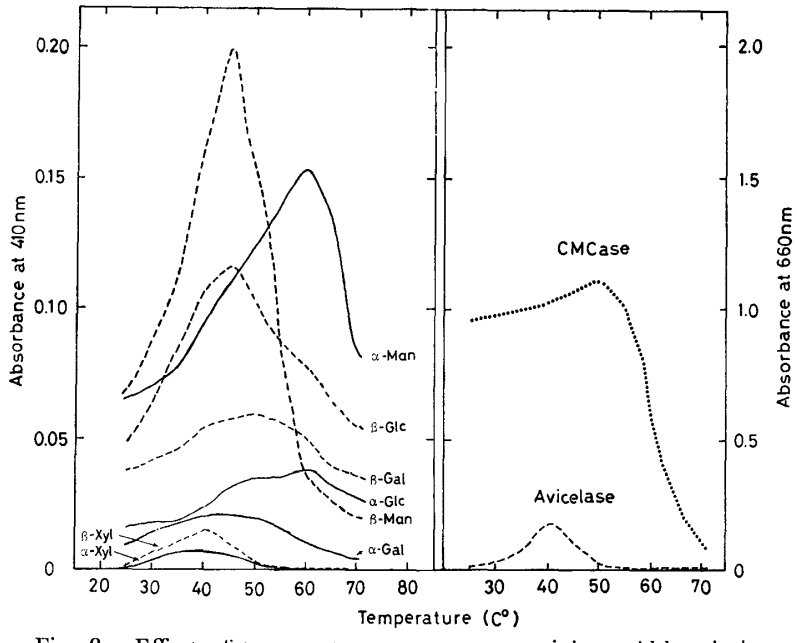

Fig. 8. Effect of temperature on enzyme activity. Abberviation was the same as shown in Fig. 2.

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